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FORTIFICATION OF RICE CRACKER MADE FROM BROWN RICE FLOUR AND BLACK RICE FLOUR WITH PROTEIN FROM OYSTER AND MUSSEL POWDER

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Abstract

FORTIFICATION OF RICE CRACKERS MADE FROM BROWN RICE FLOUR AND BLACK RICE FLOUR WITH PROTEIN FROM OYSTER AND MUSSEL POWDER

by

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Rice crackers popularity has been rising in recent years, largely due to increase in demand for gluten-free snacks. Even though rice crakcers are generally considered to be healthy snacks, over-consumption of rice crackers might lead to high glycemic responses, due to the fact that most of rice crackers in the market are using polished white rice flour. Therefore, in this experiment, brown rice and black rice flour are used to create the rice crackers. Another popular trend in food industry is the fortification process, which improve the nutritious quality of certain product. Oyster and Mussel Powder are two potential candidate that could be used to increase the antioxidant properties of rice crackers, at the same time reducing their glycemic responses. To evaluate the effectiveness of this fortification process, Glyceamic Glucose Equivalent (GGE) Assay is implemented in this experiment, along with *in vitro* starch digestion process. While for the assessing the antioxidant properties, Total Phenolic Content assay and Ferric-Reducing Antioxidant Power (FRAP) assay is used. Fortification process also influence other aspect such as texture, appearance, functional properties, pasting properties, which will be analysed in this study. The result indicates that brown rice flour shows lower GGE value compared to black rice flours, while the fortification process do affect the GGE value of rice crackers, but it only shows significant effect on brown rice crackers. In terms of Antioxidant properties, black rice crackers shows superiority than brown rice crackers, while the fortification by oyster and mussel powder process shows improvement on both brown and black rice crackers.

Keywords: **Rice Crackers, Black Rice, Brown Rice, Oyster, Mussel, Fortification, GGE, Antioxidant, Pasting, Texture Analysis, Funtional Properties**

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Chapter 1

Literature Review

1.1 Introduction

Cracker is considered one of the most popular snacks in the world. The definition of crackers itself is a thin-crisp snack produced by unleavened dough (Shukla, 1994). There is a different iteration of crackers, depending on their main ingredients or how they are processed. In western culture, more common ingredients for crackers are using wheat flour. However, in Asia, it is more common to use rice as the main ingredient for crackers. Which makes sense since the staple food in Asia is rice, while western culture might consume bread more often than rice. The cracker that utilized rice flour as their main ingredient is called rice crackers. The popularity of rice crackers is increasing in western culture due to the fact that the awareness and demand for gluten-free baking products are increasing (Xu, Zhang, Wang, & Li, 2020). Rice flour itself does not form gluten when processed into rice crackers because they do not possess the protein responsible for gluten formation. Therefore, rice crackers are suitable to be consumed by people who have celiac disease, which refers to people unable to consume gluten-containing product (Green & Jabri, 2003). Besides being gluten-free, there are other claims that rice cracker is healthier than wheat crackers based on their nutrition content. Rice crackers are believed to contain less fat compared to wheat crackers, which makes them suitable for people susceptible to obesity and cardiovascular disease (Wanyo, Chomnawang, & Siriamornpun, 2009). However, rice crackers are not without flaws and disadvantages when compared to wheat crackers. The gluten in baking products has a role in the textures of the product; therefore, most gluten-free baking products are inferior in terms of textures. In the case of crackers, the gluten inside wheat crackers is responsible for creating the puffy textures, while in the case of rice crackers, the end product does not possess this trait (Nikolaids & Labuza, 1996).

Despite the health benefits possessed by rice crackers, there is always room for improvements. There are many attempts to create better and more nutritious rice crackers. One of the most effective methods is to replace the type of rice flour used for the main ingredient. The more common type of rice flour used today is polished white rice, which is understandable because polished white rice is preferred by most people in terms of sensory characteristics. However, the popularity of using brown rice flour is increasing. The definition of brown rice itself is rice grain that still contains the outer bran layer, which gives the rice grain a brownish color. This outer bran layer is rich in nutrition, such as fiber and antioxidants (Saleh, Wang, Wang, Yang, & Xiao, 2019). Besides brown rice, there are other choices for replacing polished white rice, which is pigmented rice grains. Pigmented rice category consists of many varieties; among them are black rice, purple rice, and red rice. This pigmented rice

has been considered to be a healthier alternative to the polished white rice due to its high antioxidant properties (Saikia, Dutta, Saikia, & Mahanta, 2012). One pigmented rice, called black rice, has been rising in interest these days due to its richness in anthocyanins, which is a phenolic compound that is responsible for its black color (Khoo, Azlan, Tang, & Lim, 2017). Unfortunately, the implementation of this rice variety to food products has not been widely adopted.

Another option to increase the nutritious value of rice crackers is through the process of fortification, which means adding other ingredients with the objective to improve its nutritious value. There are several research that have been done in this field, one example is using Philippines local crops called Mallunggay to fortify the rice crackers, which leads to an increase in beta-carotene and also vitamin C content (Morales, Mnaois, Abilgos-Ramos, & Aquino, 2012). Green tea leaves have also been added to rice crackers to enhance their antioxidant capabilities (Radočaj, Dimić, & Tsao, 2014). A newer trend called upcycling is also adopted in the field of rice cracker fortification. Apple pomace, which is the waste from apple juice or puree production, has been utilized for the fortification of rice crackers. The result is an increase in several nutritious properties such as total dietary fiber and antioxidant activity. (Mir, Bosco, Shah, Swaminathan, & Mir, 2015). All the examples above are utilizing plant-based fortification; however, there is a potential candidate besides plant-based food, which is using seafood powder, more specifically oyster and mussel powder. These two ingredients contain many health benefits, including removing free radicals from the body, and they also possess antiviral and anti-inflammatory properties (Khan & Liu, 2019). However, the implementation of oyster and mussel powder in food products, especially in bakery, is extremely scarce, due to the off flavor produced by these ingredients (Klunklin & Savage, 2018)

With many approaches to improve the existing formula of rice crackers, sometimes it is difficult to decide which method gives out the most optimum result. Because of the complexity of food matrixes, sometimes adding or replacing one ingredient does not produce the expected result. With the addition of the complexity of our digestion system, it becomes more difficult to determine if the nutrition in the food is absorbed properly by the human body. Therefore, this part of literature review will further discuss how the ingredients would affect the nutritious aspect and the physical properties of the crackers.

1.2 Nutritional Qualities and Digestibility

1.2.1 Brown Rice

The awareness to consume brown rice instead of polished white rice comes from the numerous health benefits brown rice provide. The outer bran layer of brown rice is the component that responsible for these benefits. There are two ways of how this outer bran layer contributes to the health-promoting effects of brown rice. First is due to the nutritional components in the bran itself, and the second is how the existence of the bran layer affect the digestion during the consumption of

brown rice. The outer bran layer is rich in several important nutrients, among them are fiber, protein, lipids, and also minerals, such as zinc, sodium, and potassium (Wu, Yang, Touré, Jin, & Xu, 2013). Most of the lipids in rice grain are concentrated into the bran layer; however, it is important to note that the lipid content in rice is unsaturated fatty acids, which possess health-promoting effects. Oleic acid and Linoleic acid are two examples of unsaturated fatty acids that are present in rice bran (Cho, Kim, Lee, Kang, & Lee, 2006). One of the most important aspects of brown rice that has been recognized by the majority of people is the high dietary fiber content, which has been proven to be beneficial to human health. The benefits of consuming high fiber-containing foods are ranging from preventing diabetes, cardiovascular disease, and even cancer (Kaczmarczyk, Miller, & Freund, 2012). The mechanism of how fiber treating several health problems such as diabetes or obesity is believed to be connected to the gut microbiota. The fiber that the human body consumed will not be digested or absorbed readily during the digestion process, which eventually will be consumed by microorganisms in the gut. This leads to an increase in the diversity of gut microflora and eventually promote good health (Makki, Deehan, Walter, & Bäckhed, 2018). Sheflin et al. (2017) conducted an experiment where rice bran was supplied to colon cancer survivors, and the result indicates there was an improvement to the gut microbiota of the subjects.

An experiment conducted by Ohtsubo, Suzuki, Yasui, and Kasumi (2005) indicated that the outer bran layer of brown rice also contains a high concentration of gamma-aminobutyric acid (GABA), thus indicates one other aspect of rice bran that is crucial, which is the antioxidant activity it possesses. Inside the bran layer, there are numerous bioactive compounds present, such as tocopherol, GABA, andγ-oryzanol; however, which bioactive compound is the dominant one is extremely difficult to determine in brown rice. One of the reasons for this is due to the variety of bioactive compounds present depending on its genotype and environment (Saleh et al., 2019).

As mentioned above, the bran itself is full of nutritious components, but they also provide a protection mechanism that affects the digestion process when compared to polished white rice. The digestibility of starch of white rice often becomes a concern to many people due to its faster rate of starch digestion, which leads to high glycemic response. In contrast, brown rice possesses the outer bran layer, which itself delayed the rate of starch digestion, thus producing a lower glycemic response compared to white rice (Atkinson, Foster-Powell, & Brand-Miller, 2008). The connection between glycemic responses with several health complications has been strongly established, and it is believed that consuming high glycemic index food might increase the risk of diabetes (Sun et al., 2010). The lower glycemic response of consuming brown rice is also affected by the gastric emptying rate. It is believed that the tougher physical characteristic of brown rice creates a slower rate of gastric emptying when compared to white rice (Pletsch & Hamaker, 2018). Some evidences suggest a slower rate of gastric emptying may be responsible for affecting the appetite and satiety feeling,

which might benefit in lowering food consumption in general (Clegg, Ranawana, Shafat, & Henry, 2013).

1.2.2 Black Rice

Black rice (*Oryza sativa* L.) has been increasing in popularity in recent years. Originated from Asian countries, especially Thailand and China, now it has been consumed around the world. The application of black rice has been broadened; due to its nutty flavor, it can be used to create a variety of baking products and beverages (de Lima et al., 2018). This increase in consumption is largely caused by its health benefits, especially its antioxidant properties. One bioactive compound called anthocyanin is responsible for the dark pigment of black rice, which is accountable for the high antioxidant activity of black rice. Anthocyanin is categorized as a type of flavonoid, and its chemical structure makes them capable of reacting with Reactive Oxygen Species (ROS), which is considered to be toxic for the human body (Bueno et al., 2012). Consuming a proper amount of anthocyanins has been proven to improve several health traits, such as cancer, diabetes, and cardiovascular disease (Hu, Zawistowski, Ling, & Kitts, 2003).

In comparison to brown rice, black rice has been proven to be superior in terms of phenolic compound concentration (Ziegler et al., 2018). One notable difference is for brown rice, bioactive compounds lie mostly on the bran layer, while for black rice, most of the anthocyanin lies on the aleurone layer of rice grain (Lee, 2010). In other words, even after the bran layer of black rice has been removed, it still possesses high antioxidant capabilities. When compared in terms of digestibility, it is complicated to determine which of these two-rice varieties would excel each other. The previous section stated that the bran layer of brown rice is the one that provide integrity and structure to retard the rate of digestion. While for black rice, it is believe that anthocyanin might be the one responsible for slowing the rate of starch digestion by inhibiting the digestive enzyme (McDougall, Dobson, Smith, Blake, & Stewart, 2005). It also has been confirmed that adding anthocyanins extract during starch digestion might increase the concentration of resistant starch (RS), which further hinders the rate of starch digestion (Camelo-Méndez, Agama-Acevedo, Sanchez-Rivera, & Bello-Pérez, 2016). However, the conclusion is still unclear. Some would argue that the anthocyanin and other phenolic compounds in rice would be depleted when it reaches the digestive tract, thus rendering them inactive (Thuengtung, Niwat, Tamura, & Ogawa, 2018). The complexity of food matrices also responsible for a further complication of this matter. It is believed that approximately 80% of phenolic compounds in rice belong to the bound phenolic group, which means it will not be released until it reaches the colon during digestion. While in contrast, free phenolic compounds is released in small intestine, where it is easily absorbed by the body (Acosta-Estrada, Gutiérrez-Uribe, & Serna-Saldívar, 2014). The form of which the rice is consumed will affect the

number of phenolic compounds that can be released during digestion process; thus, it is extremely difficult to determine the effectiveness of black rice in slowing the rate of starch digestion.

1.2.3 Mussel

As mentioned above, the other method to improve the nutritious value of crackers is through the fortification process. Mussel powder has been proposed to contain numerous health benefits. However, due to its strong off-flavor, their application on different food products is still incredibly limited (Klunklin & Savage, 2018). It is believed that the off-flavor is produced through the process of lipid oxidation. The use of Nutmeg and Cinnamon has been suggested to reduce this off-flavor due to their nature as antioxidants, which could reduce lipid oxidation (Su et al., 2007). The high interest in oysters and mussels lies in the health benefits they provide, more specifically is their anti-inflammatory effect. For mussel, this health benefit comes from the high concentration of Omega-3 polyunsaturated fatty acid inside the mussel (Treschow et al., 2007). Lyprinol is one example of a dietary supplement that has implemented the lipid extract of green-lipped mussel to become an anti-inflammatory product (U. Grienke, J. Silke, & D. Tasdemir, 2014) (Ulrike Grienke, Joe Silke, & Deniz Tasdemir, 2014). Mussel also possesses tremendous antioxidant properties, as proven by research conducted in Korea, where mussel demonstrated radical scavenging activity and also hypertension lowering capabilities (Jung et al., 2007). During in vitro digestion, mussel would release these antioxidant peptides, which will react with ROS; therefore, it is important to note that these antioxidants could survive the digestion process. Asides from the health-promoting effect, the antioxidant properties of mussels also have the potential of improving the quality and shelf life of food product, due to its effect in retarding lipid peroxidation, which could decrease the shelf life of certain food product (U. Grienke et al., 2014).

The addition of Mussel powder into food product also has an effect on its digestibility. Klunklin and Savage (2018) experiment showed that biscuit fortified with mussel powder exhibited lower Glycemic Index (GI) compared to an unfortified biscuit. The mechanism behind this phenomenon might be related to the protein-starch interaction that happened inside the biscuit. The protein from mussels would interact with starch from biscuit and form a matrix which would provide a protection for starch during digestion process, thus lowering its rate of digestibility (J. Singh, Dartois, & Kaur, 2010). Although there might be an alternative hypothesis, which is the interaction between bioactive compounds and digestion enzyme. As mentioned in the previous section, bioactive compounds such as anthocyanin possess the capabilities of inhibiting the digestive enzyme, which could affect the rate of digestion (McDougall et al., 2005). However, it is still unclear if this is the case, further experiment regarding this issue needs to be conducted.

1.2.4 Oyster

In terms of nutritious quality, oysters and mussel both have comparable properties. Both contain bioactive compounds, high in protein, lipids, and minerals (Khan & Liu, 2019). The lipids contained in oyster also has similar Omega-3 PUFA with mussel, which makes them possess similar anti-inflammatory properties with mussel (Dagorn et al., 2016). In terms of determining which one is superior to the other is a challenging task to overcome. The research regarding this topic is still very limited, and it is too early to get a conclusive answer. However, it is an undeniable fact that each genotype and the habit of where these marine mollusks will affect their nutritious value.
One notable difference between oysters and mussel might be lies in the food application in the market. Oyster containing products have been common in the food market. One particular product is the fermented oyster sauce. This product itself has been proven to contain bioactive compounds from oysters, which benefit in lowering hypertension (Wijesekara & Kim, 2010). However, the implementation of oyster powder into baking products still considered to be scarce. Therefore, it is difficult to compare the effect of adding the oyster powder to the rate of starch digestibility.

1.3 Physical Effect on Crackers

All the above information has been solely focusing on the nutritious quality and effect on the digestibility of the materials, but it is crucial to investigate their effect on the physical properties of the crackers themselves. Substitution or addition of certain ingredients will exert some alterations to a certain product. It is essential to determine the intensity of these modifications. In this section, the effect of each ingredient on the physical properties of crackers will be discussed in detail.

1.3.1 Brown Rice

In theory, the effect of brown rice on the textural properties of rice crackers should have a positive correlation with the hardness of the crackers. It is explainable through the texture of the brown rice itself. The bran layer of brown rice would provide extra structure on the rice grain, which makes it harder in texture. It has been proven in terms of cooked rice, where cooked brown rice possesses a harder texture than white rice (Ziegler et al., 2018). However, in the field of baking products, the physical form of brown rice that is utilized is in flour form, where the original structure of rice grain has been destroyed. The interaction between brown rice flour and other ingredients such as shortening and water also has an effect on the end result. Baek and Lee (2014) conducted an experiment where brown rice flour is incorporated into pasta dough, and the result showed that the product possesses lower tensile strength and becomes easier to break down. While in other experiment involving bread, the use of brown flour has been proven to create a network of thinner filament when compared to using white rice; although both of them are still inferior compared to use

of wheat flour (Nikolić, Dodić, Mitrović, & Lazić, 2011). As mentioned in the previous section, the rice bran layer contains most of the lipid content of rice grain, which makes brown rice to be containing higher lipid concentration than polished white rice. This high lipid content might also contribute to lowering the hardness of the crackers produced. The lipid itself is considered as hydrophobic; thus lipid-containing flour would acquire a lower Water Absorption Index (WAI), which could lead to producing less structural dough (Alcázar-Alay & Meireles, 2015).

1.3.2 Black Rice

Incorporating black rice into baking products also induces certain effects on the physical properties of the product. The bioactive compound inside black rice, specifically anthocyanin, might interact with food matrices during dough formation. An experiment conducted by Sui, Zhang, and Zhou (2016) indicated that anthocyanin extract influences weakening disulphide bond during dough formation, which leads to poor gas retention of and tighter structure. This phenomenon leads to an increase in hardness for bread fortified by anthocyanin extract. In contrast, the elasticity, springiness, and cohesiveness of fortified bread are reduced due to the weaker gluten network. However, this is in terms of bread, which contains a gluten network. For rice cracker, which is gluten-free, the way of how black rice affecting texture might arise from a different approach. The carbohydrate content, especially amylose, has an effect on the crispiness and hardness of cookies through its influence on creating a three-dimensional network of structure (Wang et al., 2016). Black rice is believed to contain a lower total of carbohydrate and amylose content compared to normal unpigmented white rice, which could lead to lower hardness cookies to be produced (Kraithong, Lee, & Rawdkuen, 2018). Nevertheless, the amount of research using black rice for making rice crackers is still inadequate; therefore, it is difficult to draw a conclusive result.

Even though the incorporation of these varieties of rice flour always proposes a modification to the physical properties of crackers, it is possible to control or minimize these changes. Various methods of pre-treatment for the rice flour might affect the intensity of these physical alterations. Structure modification, such as grinding of the rice flour into distinct sizes of granules, would impose different properties for the crackers (Dhull, Punia, Kumar, Singh, & Singh, 2021). Other than a physical modification to rice flour, heat treatment also affects the cooking properties of rice flour, which might lead to different structural properties of crackers (Lang et al., 2020). Germination and other chemical modification also have been proposed to be a possible method to alter and control the effect of substituting rice flour in crackers.

1.3.3 Mussel and Oyster

The addition of green-lipped mussel powder into crackers shows a predictable effect. The mussel powder is rich in protein and fiber, which when incorporated into the dough, will eventually absorb

more water. This will create an increase in hardness of crackers due to lower moisture content (Klunklin & Savage, 2018). It is also applicable in terms of gluten-free bread fortification with mussel powder, where the addition of mussel powder would increase the firmness of the bread (Vijaykrishnaraj, Roopa, & Prabhasankar, 2016). However, it is important to note that the increase in textural integrity is followed by the development of green-dark color and strong off-flavor to the product. These traits often propose a problem that could be minimized by adding other ingredients, such as nutmeg or cinnamon (Su et al., 2007). In terms of oysters, due to their resemblance in structure and content with mussel, the result of incorporating them into crackers is expected to produce a similar result.

Chapter 2

Objective and Hypothesis

2.1 Aim and Objective

The aim for this experiment is to determine whether changing the type of rice flour in rice crackers would impose significant changes. Other than altering the rice flour, we also evaluate the effects of fortification with oyster and mussel powder. The assessment that we perform focused on both the physical characteristic, functional properties, pasting properties, starch digestibility, glycemic responses, and antioxidant properties.

2.2 Hypothesis

Based on preliminary research, the hypothesis of this experiment are as follows:

1. The inclusion of brown rice would improve the nutritious value of rice crackers, such as total dietary fiber, lipid content, and protein content. The use of brown rice flour would also reduce the glycemic responses and starch digestibility of rice crackers, as well increase its antioxidant activities.
2. The usage of pigmented rice, in this case black rice, would increase the nutritious value of rice crackers, especially its antioxidant activities. The bioactive compounds in black rice also possess the ability to reduce the glycemic responses and rate of starch digestibility for the rice crackers.
3. Replacing white rice with brown rice and black rice will have impact on the physical characteristic of the rice crackers. The rice crackers made from brown rice might have lower hardness, while the use of black rice might increase the hardness of rice crackers.
4. Fortification of rice crackers with oyster and mussel powder would increase the hardness of the rice crackers, due to decrease in moisture content. However, oyster and mussel powder have a positive effect in improving the antioxidant properties of the rice crackers and reducing the glycemic responses.

Chapter 3

Material and Methods

3.1 Raw Material

Both brown rice and black rice are imported from Thailand. While oysters and mussels powder is purchased from Aroma (NZ) Ltd. The other ingredients required for rice crackers preparation, such as sugars (Chelsea®), salts (Pams®), baking powder (Pams®), cooking oil (Pams®), Xanthan gum (Lotus®), Lecithin granules (Lotus®), were purchased from local supermarket New World®, New Zealand.
The rice flour is obtained by grinding the rice grain using a laboratory sized miller (Laboratory Mill 3310). Rice grains are poured into the miller with the setting of the smallest granules size (0 settings). The process is repeated several times to ensure the granule sizes are uniform. After the milling process, the flour is pass through a 500 µm sized sieve to separate any impurities or large-sized granules. The rice flours are then stored in a sealed bag at room temperature.

3.2 Rice Cracker Preparation

The recipe and method for rice crackers preparation are based on the recipe by Mir et al. (2015) with several modifications to suit this experiment. One major difference is that we use the addition of oyster and mussel powder instead of substitution. Therefore the water volume of fortified crackers needs to be adjusted as well. The second adjustment is that we only evaluate one concentration of shellfish powder, which is 20% of the total rice flours used. The complete recipe is listed in **Table 1**; basically, the recipe would include 180 grams of rice flours (either brown or black), 36 grams of shellfish powder (either oyster or mussel), 68.4 grams or 98.4 grams of waters (higher amount of water for fortified crackers), 12 grams of sugar, 3 grams of baking powder, 1.8 grams of salt, 1.8 grams of xanthan gums, 1.8 grams of lecithin granules, 7.2 grams of maltodextrin, and 24 grams of cooking oil.
The process of making the crackers started with the mixing of dry ingredients, except for sugar, in a mixing bowl. Rice flours, oyster or mussel powder, salt, and baking powder are mixed using a whisk to ensure homogenous blending. The weighing process for all ingredients utilizes a digital weight scale with the accuracy of two decimal points. In a separate container, mix oil, water, xanthan gum, lecithin granules, maltodextrin, sugar, and water to create the emulsion. The blending process for the emulsion is done using a stand mixer (Delta Food Equipment®). Mixed all the emulsion ingredients for ten minutes using high-speed settings to ensure all ingredients are combined homogeneously. Then slowly pour the combination of dry ingredients into the emulsion and continue the mixing

process for five minutes using a low-speed setting until the dough is formed. After that, remove the dough from the stand mixer and form them into one clump, which will be stored in a sealed bag for 30 minutes for the resting process. This resting process is essential to make sure the dough is incorporated together. Then roll the dough using a rolling pin until it reaches two-millimeter thickness. It is important to ensure the thickness is uniform across the dough. Cut the dough into uniformly sized round crackers using a 5-centimeter diameter cutting mold. Place the rolled dough into a baking tray and put it into a preheated oven. The oven is set to 150^{o} Celsius temperature. The rice crackers are baked for about six minutes, then take it out from the oven. The next step would involve flipping the crackers, then put them back into the oven for another six minutes of baking time. This will ensure both sides of the crackers are cooked evenly. The last step would involve letting the crackers cool down before storing them into a sealed plastic bag, which will be stored for further use. The rice crackers are stored at room temperature for the first week, and then they will be transferred into the freezer for a longer storage time.

Table 1. Composition of Rice Cracker

Ingredients	Brown 100%	Brown +20% Oyster	Brown + 20% Mussels	Black 100%	Black+20% Oyster	Black +20% Mussels
Brown Rice Flour	180 g	180 g	180 g	-	-	-
Black Rice Flour	-	-	-	180 g	180 g	180 g
Oyster/Mussel Powder	-	36 g	36 g	-	36 g	36 g
Water	68.4 g	98.4 g	98.4 g	68.4 g	98.4 g	98.4 g
Sugar	12 g	12 g	12 g	12 g	12 g	12 g
Baking Powder	3 g	3 g	3 g	3 g	3 g	3 g
Salt	1.8 g	1.8 g	1.8 g	1.8 g	1.8 g	1.8 g
Lecithin	1.8 g	1.8 g	1.8 g	1.8 g	1.8 g	1.8 g
Xanthan Gum	1.8 g	1.8 g	1.8 g	1.8 g	1.8 g	1.8 g
Maltodextrin	7.2 g	7.2 g	7.2 g	7.2 g	7.2 g	7.2 g
Cooking Oil	24 g	24 g	24 g	24 g	24 g	24 g

3.3 Physical and Texture Analysis

3.3.1 Dimension of Crackers

The thickness and width of the crackers are evaluated for analyzing the dimension of the crackers. Vernier Caliper is used to measure the thickness and diameter of the crackers. In total, twenty crackers in a sample group are selected for measurement. For both thickness and diameter, for each cracker, two measurements are recorded at a different location with an approximately 90-degree

angle apart. Measurements are logged in millimeters. To obtain spread ratio, simply divide diameter with thickness.

3.3.2 Weight of Crackers

The weight of the crackers is assessed using a laboratory weighing scale with three decimal point accuracy. The weight of twenty crackers for each sample group are recorded in grams.

3.3.3 Colour Analysis

For recording the color, Colourimeter (Konica Minolta, Chromameter CR-400, Osaka, Japan) is used. Colour space that is measured consists of L* (lightness), a* (redness), b* (yellowness). There are two forms of samples that need to be analyzed, first is the sample in fully intact cracker form, and the other is the sample in powder form. In order to achieve the powder form, samples are grounded using commercially available food and coffee grinder (Breville®). Then the powder will be pass through a 500-micron sieve to remove any large particles. For measuring the color in cracker form, ten crackers from each sample group are chosen, and for each cracker, three different readings from three separate locations are taken. While for powdered form, the sample would be laid out on a piece of white paper, and five different readings from various positions are recorded. Prior to measuring each sample, the colorimeter is required to be calibrated using standardized white tiles.

3.3.4 Moisture Content

For determining the moisture content, we utilized the oven drying method. The first step would be weighing the metal canister, and it is important to use a weighing scale with the accuracy of three decimal points with a glass cover to reduce the error during the weighing process. For the metal canister, it is essential to dry them in the over prior to using them to eliminate traces of moisture. Weight the empty metal canister, then put approximately 3.5 grams of each sample into the canister. Each sample would require three replicates. Finally, place the sample with the metal canister into the oven (190^{o} Celsius) overnight or at least 6 hours. After the drying process, remove the samples from the oven and cool them down inside the desiccator for 10 minutes before the weighing process.

3.3.5 Texture Analysis

The hardness and fracturability of the crackers are measured using Texture Analyzer (Stable Microsystem, TA XT Plus) with a five kilograms load. The method of measuring these two properties is based on method by Adeola and Ohizua (2018) with slight modification. A three-point bend rig probe is chosen for this analysis, which is a common probe for measuring biscuits or crackers. Single-cycle measurement is adapted in this test to simulate the first bite during consumption. When performing the test, twenty crackers from each sample group are selected to be measured. The

graph of force-time resulted from this test are analyzed by texture analyzer software to produce hardness (grams) and fracturability (millimeters) data for all samples.

3.4 Pasting Properties

For evaluating the pasting properties of all samples, we utilize Rapid Visco Analyzer (RVA Super 4, Newport Scientific®). Samples will be evaluated in both flour and cracker states. The powdered cracker samples are obtained from grinding the crackers, as explained in the Colour Analysis section. This process will adapt Adeola and Ohizua (2018) methodology with slight modification in slurry preparation. Both flour and cracker samples are measured. Powdered samples are mixed with water to create a slurry, which will be analyzed by the RVA. The weight of the samples and water are varied, depending on the type of the samples. For pure rice flour sample, 4 grams of flour is mixed with 25 grams of water. While for flour and shellfish powder blend, we use 3.2 grams of rice flours and 0.8 grams of shellfish powder mixed with 25 grams of water. For crackers samples, 4 grams of powdered crackers are mixed with 25.28 grams of water. This variation of water volume is due to the difference in moisture content between flour sample and crackers sample.
The slurry mixture inside the canister will then be stirred for 1 minute at 160 rpm; then, the temperature will be increased to 50^{o} Celsius, where it will be held for a minute. Later, the temperature will be increased again until it reaches 95^{o} Celsius for another five minutes. Finally, the slurry will be cooled down back to 50^{o} Celsius gradually for approximately 7 minutes, where it will be held at this temperature for another 2 minutes. The viscosity during this process will be recorded for analysis, which will produce data on peak viscosity, pasting temperature, setback viscosity, and final viscosity. For each sample, at least a triplicate of data will be produced and analyzed.

3.5 Functional Properties

3.5.1 Swelling Capacity

The method for assessing the swelling capacity of all flours samples is based on the methodology developed by Sosulki, Humbert, Bui, and Jones (1976). The flour sample is poured into a 100 ml measuring cylinder until it reaches 10 ml mark. Then fill the measuring cylinder with distilled water until it reaches 50 ml mark. The top of the measuring cylinder is sealed tightly using paraffin film, then the water and flour inside the cylinder are mixed by inverting the cylinder two or three times depending on the solubility of the mixture. After two minutes have passed, the suspension needs to be inverted again, then let it stand for another eight minutes. In the end, the final volume occupied by the flour sample will be recorded.

3.5.2 Water Absorbtion Capacity (WAC)

The method developed by Sosulki et al. (1976) for measuring water absorption capacity for flour is used for this experiment. Measure one gram of the flour sample using a digital weighing scale, then place the sample into a 15ml falcon tube. Then add 10 ml of distilled water into the falcon tube. Place all of the falcon tubes into a rack, and it is important to ensure all falcon tubes are vertically upright. Let them stand at room temperature for 30 minutes. The tubes were then required to be centrifuged for 30 minutes at the speed of 3000 rpm (Rotina 360, Hettick Zentrifugen ®). Remove the tubes from the centrifuge, then remove the supernatant leaving the solid sediment behind. The last step would require weighting the tube with the solid residue and record the data to calculate the weight of the water absorbed by the flour.

3.5.3 Oil Absorbtion Capacity (OAC)

For measuring oil absorption capacity, the methodology would be similar to measuring water absorption capacity. The only difference would be using cooking oil instead of distilled water. The other important thing to note is for oil absorption capacity when removing the supernatant after centrifuge, it is essential to invert the tubes for a prolonged period of time (approximately 3 minutes) to ensure all the oil is removed.

3.6 Total Starch Analysis

The method that we adapted to determine the total starch content is the RTS-NaOH procedure, which is adapted because our samples contain resistant starch. The steps for this method is as described by McCleary, Charmier, and McKie (2019) with minor modification. The first step would involve weighing 100 mg of the samples in duplicate and transfer them into Corning culture tube. The next step is essential to ensure complete dissolution of the sample with high starch content. It requires the addition of 0.2 ml of 80% ethanol and uses vortex mixer to disperse the sample. Then using 5 ml pipette, accurately add 2 ml of 1.7 M sodium Hydroxide solution and mixed them using the vortex mixer for 15 seconds. Place small magnetic stir into each tube and place them over magnetic stirrer for 15 minutes. During the course of 15 minutes, it is important to mix them using a vortex mixer three separate times to ensure complete dispersion of the sample. Then place 8 ml of 0.6 M Sodium Acetate Buffer pH 3.8 into each test tube using a 10 ml pipette, subsequently add 0.1 ml of thermostable alpha-amylase and 0.1 ml of Amyloglucosidase enzyme (3300 U/ml). Vortex the tubes for 5 seconds to ensure a complete mixing process. Place the test tubes into a 50^{o} C water bath for about 3 minutes. Later take out the test tubes from the water bath and allow them to cool down before inverting them three times to mix the condensed water alongside the tube with the sample.

After the test tube has been completely cooled down, take 1.6 ml of each aliquot, and transfer them into the Eppendorf tube, where they will be centrifuged at 13000 rpm for 5 minutes. Then transfer 1000 µL of the supernatants into glass tubes containing 4 ml of 0.1 M acetate buffer pH 5.0. Mix the content, then transfer 0.1 ml of the solution into another glass test tubes, where 3.0 ml GOPOD reagent will be added. In another test tubes, prepare 0.1 ml of 1.0 mg/ml glucose standard solution in triplicate and add 3.0 ml GOPOD reagent. This will act as references value. Incubate all the samples and glucose standard solution in 50^{o} C water bath for another 20 minutes. Finally, read the absorbance of all samples at 510 nm using a spectrophotometer and record all the data.

3.7 Glycemic Glucose Equivalent (GGE)

3.7.1 *In Vitro* Starch Digestion

For evaluating the amount of reducing sugar released over the course of two hours of digestion, we adopted the methodology described by Foschia, Peressini, Sensidoni, Brennan, and Brennan (2014). Using a digital weighing scale, measure 2.5 grams of powdered samples (flours and crackers) into a plastic biopsy pot. It is important to keep one pot without sample to act as blank. Then add 30 ml of distilled water into the pot using a bottle-top dispenser. Place the biopsy pots into 15 sized magnetic multi-stirrers (RT 15 power, IKA®-Werke) and add 0.8 ml of 1 M Hydrochloric Acid into each container using 1 ml pipette to simulate acidic condition during gastric digestion. Then increase the temperature until the sample reaches 37^{o} Celsius. It is important to keep the temperature between 35^{o} and 40^{o} Celsius throughout the process. When the temperature reaches 37^{o}, add 1 ml of 10% pepsin solution (Using 0.05M HCL) into each container. Cover the samples with tinfoil sheets and a towel to prevent heat loss and keep the temperature within the range. Hold the 37^{o} temperature for 30 minutes to let the sample be digested. The next step would require the addition of 2 ml Sodium Bicarbonate ($NaHCO_3$) solution into each pot, which will be followed by adding 5 ml of 0.1 M sodium Maleate buffer pH 6. Then take 1 ml aliquots from each pot and place them into 15 ml falcon tubes containing 4 ml of ethanol, which will halt any reaction in the sample. These aliquots will serve as time 0 of the digestion process. Next, add 0.1 ml of amyloglucosidase enzyme into each pot, followed by the addition of 5 ml 2.5% pancreatin solution (Using Sodium Maleate buffer) into each sample container. This will mark the start of the digestion process, then add 10 ml of distilled water to each pot to make the volume of each container to be exactly 53 ml. When the digestion time reaches 20 minutes mark, take 1 ml aliquots, and put into 15 ml falcon tubes containing 4 ml of ethanol. Repeat this step at 60 minutes and 120 minutes mark of the digestion process. Store all the samples in the falcon tubes into the freezer for further use.

3.7.2 Reducing Sugar Measurement

The samples from *in vitro* digestion will undergo a centrifuge process at 1000 rpm for five minutes. It is important to note that if after the centrifugation process the sediment and supernatant do not show complete separation, repetition of centrifugation is required. Take 50 µL of the supernatant and transfer it into a glass test tube, followed by adding 50 µL of distilled water into the same test tube. In a separate test tube, prepare 5mg/ml and 10 mg/ml glucose standard solution, which will be used as reference value for quantification of reducing sugar. Then add 0.25 ml of enzyme solution A (1% invertase, 1% amyloglucosidase in acetate buffer pH 5.2) into each glass test tube. Let the sample digest for at least 20 minutes at room temperature. After that, add 0.75 ml of DNS mixture (0.5 mg/ml glucose, 4 M NaOH, DNS reagent, in a ratio of 1:1:5 respectively) into each test tube. Place the test tube into a water bath with a temperature of 95^{o} Celsius for 15 minutes. It is vital to cover the test tube with aluminum foil to prevent the water steam to drip into the test tube. Then remove the test tube and let it cool down for 5 minutes before measuring its absorbance. Set the spectrophotometer to a 530 nm wavelength and calibrate using a cuvette filled with distilled water. Measure the absorbance for all the samples and record the data for further analysis.

3.7.3 Area Under Curve (AUC)

To evaluate and compare the glycemic response of the samples, the AUC method is used. After obtaining the absorbance data from the previous step, quantification of reducing sugar need to be done using a calculation based on reference values of standard glucose solution. Then the graph of reducing sugar released against the time of digestion need to be constructed for all samples. Using the trapezium method, calculate the approximate AUC value, which then will be compared among all samples.

3.8 Antioxidant Assay

3.8.1 Extraction

The extraction process for antioxidant components will be done using 50% methanol solution. First, weigh 1 gram of sample and place them into a plastic biopsy pot. Place all pots into a multi-magnetic stirrer and stir for at least 3 hours at room temperature. Then transfer the sample extract to falcon tubes and centrifuge at 2500 rpm for 10 minutes. Take 1.5 ml of the supernatant and place them into the Eppendorf tube. Store the sample at -20^{o} C until further use.

3.8.2 Total Phenolic Content (TPC)

The method that has been chosen for determining TPC is the Folin-Ciocalteu method. The first step for assessing TPC would be the preparation of gallic acid standard solution. By implementing dilution technique, prepare 0, 12.5, 25, 50, 75, 100, and 150 µg/ml gallic acid, using 70% methanol as solvent. Prepare 96-well microplate (STRATA®) and place 20 µL of sample (from methanol extraction and *in vitro* digestion extract) or standard solution, 100 µL 0.2 N Folin-Ciocalteu reagent, and 80 µL of 7.5% Sodium Bicarbonate on each well. Incubate the microplate for 2 hours at room temperature and covered it from light. Using Microplate Reader (FLUOstar Omega, BMG Labtech), measure the absorbance for each sample and standard solution at 760 nm wavelength. Analyze the data using Microplate reader software (MARS, BMG-Labtech).

3.8.3 Ferric Reducing Antioxidant Power (FRAP) Assay

The method of measuring FRAP for this experiment is adapted from a procedure developed by Benzie and Strain (1996) with some modifications, because in this experiment 96-well microplate reader is used instead of traditional cuvettes. The first step in this assay is to prepare the FRAP reagent, which should be prepared fresh on the day of the assay. Mix 300 µM Acetate buffer pH 3.6 with 10 mM TPTZ in 40 mM HCL and 20 mM $FeCl_3$ at the ratio of 10:1:1, respectively. Then prepare the standard solution of Iron sulphate heptahydrate ($FeSO_4 7H_2O$) ranging from 1000, 800, 600, 500, 300, 100, and 0 µM concentration using distilled water as the solvent. Prepare 96-well microplate, in each well place 210 µL of FRAP reagent, and 7 µL of the sample (from methanol extraction and *in vitro* digestion extract) or standard solution. Then incubate the sample for 2 hours at 37^{o} C using an incubation chamber (MIDI Dual 14, HYBAID). Then using a microplate reader, measure the absorbance for all samples at 593nm wavelength.

3.9 Statistical Analysis

All data will be processed through Minitab® Statistical software using ANOVA test to determine whether there are any significant differences between the sample groups. For multiple groups, Tukey Multiple Group test will be utilized. The p-value less than 0.05 is used to determine ifa significant difference is present.

Chapter 4

Result and Discussion

4.1 Physical and Texture Analysis

Table 2 shows that in terms of Spread Ratio, there is no significant effect on adding Oyster and Mussel powder into both black and brown rice crackers ($p<0.05$). However, for thickness, BLO sample shows a higher value compared to the other samples. This might be explained by the flaw in our experimental method. When rolling the rice cracker dough, we utilized a hand-rolling pin. Although it has thickness guidance attached to the pin, it is still proven difficult to achieved uniform thickness across the dough, moreover within dough from different crackers. Therefore, the higher thickness in BLO might have resulted from this human error. There might be a better method to roll the dough into more uniformly thickness, which is using a rolling machine such as a pasta maker, which has an adjustable thickness and could produce more uniformly dough.

Table 2. Physical Measurement of Crackers Including Width, Thickness, Spread Ratio, and Weigh and Moisture Content

Sample	Width (mm)	Thickness (mm)	Spread Ratio	Weight (g)	Moisture Content (%)
BL	51.49 ± 0.55^{c}	2.17 ± 0.33^{b}	24.28 ± 3.55^{ab}	4.63 ± 0.82^{bc}	6.88 ± 0.25^{a}
BLM	51.95 ± 0.33^{ab}	2.23 ± 0.27^{b}	23.65 ± 3.19^{ab}	4.71 ± 0.63^{abc}	11.24 ± 0.06^{b}
BLO	52.11 ± 0.31^{a}	2.55 ± 0.54^{a}	21.46 ± 5.13^{b}	5.46 ± 1.29^{a}	9.18 ± 0.11^{c}
BR	51.01 ± 0.62^{d}	1.98 ± 0.21^{b}	26.09 ± 2.81^{a}	4.07 ± 0.48^{c}	5.50 ± 0.03^{d}
BRM	51.52 ± 0.58^{bc}	2.28 ± 0.33^{ab}	23.04 ± 3.32^{ab}	4.90 ± 0.89^{ab}	8.13 ± 0.05^{e}
BRO	51.47 ± 0.44^{c}	1.98 ± 0.26^{b}	26.36 ± 3.36^{a}	4.31 ± 0.67^{bc}	7.72 ± 0.05^{f}

BL (Black Rice), BLO (Black Rice + 20% Oyster), BLM (Black Rice +20% Mussel), BR (Brown Rice), BRO (Brown Rice +20% Oyster), BRM (Brown Rice +20% Mussel)

Value: Mean±Standard Deviation

$^{a-f}$ superscripts in each column indicate significant differences ($p<0.05$)

The weight differences across samples show heavier crackers are produced when shellfish powder is added to the formula. In this experiment, it is oyster powder for black rice and mussel powder for brown rice ($p<0.05$). The shellfish powder contains a rich amount of protein content, which when will absorb more water during the mixing process. This will reduce the amount of free water that will evaporate during the cooking process, thus resulting in lower weight loss (Klunklin & Savage, 2018). There is no significant difference between the weight of black rice crackers and brown rice crackers,

which indicates the weight loss between black rice flour and brown rice flour is similar. However, the thickness of the rice crackers also has a correlation with the weight of the crackers; therefore, the explanation of the uneven thickness of the dough might also affect the weight.

Table 3. Hardness and Fractubility of Crackers Sample

Sample	Hardness (g)	Fractubility (mm)
BL	1463.12 ± 310.82[a]	24.46 ± 0.42[a]
BLO	1322.54 ± 380.21[a]	24.27 ± 0.29[ab]
BLM	1175.03 ± 471.04[a]	24.40 ± 0.58[a]
BR	772.40 ± 283.44[b]	23.92 ± 0.75[b]
BRM	1261.44 ± 419.67[a]	24.35 ± 0.45[ab]
BRO	1244.91 ± 353.61[a]	24.08 ± 0.34[ab]

BL (Black Rice), BLO (Black Rice + 20% Oyster), BLM (Black Rice +20% Mussel), BR (Brown Rice), BRO (Brown Rice +20% Oyster), BRM (Brown Rice +20% Mussel)

Value: Mean±Standard Deviation

[a-b] superscripts in each column indicate significant differences ($p<0.05$)

The texture of the sample groups is shown in **Table 3**. Hardness and Fractublity are two aspects that we have analyzed. The hardness of the crackers shows an expected outcome, which shows the hardness of black rice crackers to be higher compared to brown rice crackers. BL sample shows almost 100% increase in hardness when compared to BR sample; the result is in cohort with an experiment conducted by Sui et al. (2016), where the bread fortified by anthocyanin extract produced bread with harder texture. The explanation for this could be related to the reducing effect of anthocyanin from the black rice, where during dough formation, anthocyanin would hinder the formation of disulphide bonds (SS-SS) due to its interaction forming hydrogen bonds facilitating SH-SS linkage. Therefore, reducing the number of disulphide bonds formed. Lower disulphide bonds would result in a tighter and denser structure of bread, thus inducing an increase in hardness for the bread (Sui et al., 2016). However, it is important to understand that our experiment is using rice flour instead of wheat flour, which indicates that it does not produce a gluten network as in bread; but, the protein in rice flour also produce disulphide bonds during dough formation, which implies the theory above could also be implied in this case (Kithikorn & Hongsprabhas, 2012). The lower amount of disulphide bonds produced also responsible for the decrease in elasticity and cohesiveness due to its tight and dense structure, which also explains the increase in fractubility of BL compared to BR sample.

When compared to using brown rice flour, the hardness level between BR and BL differs significantly ($p<0.05$). The result for this low level of hardness for BR might be clarified with the fact that the bran layer in brown rice might affect the three-dimensional structure of the dough. As we know, most of the lipid content of brown rice lies on the bran layer, which when incorporated into dough, lipids would repel water and thus increasing the moisture content of the dough. This will lead to smoother and more elastic structure of crackers, thus reducing its hardness (Alcázar-Alay & Meireles, 2015). An experiment conducted by Nikolić et al. (2011) also proven that using brown rice flour instead of white rice flour would produce a thinner filament of protein network during dough formation, which explained the decrease in hardness of the rice crackers.

Fortification of Black rice crackers with shellfish powder does not show any significant effect on hardness ($p>0.05$), while fortified brown rice crackers demonstrate a substantial increase in hardness in both oyster and mussel powder addition ($p<0.05$). This increase in hardness for brown rice crackers might have resulted from an increase in crude protein and fiber content due to the addition of mussel or oyster powder. Improvement in protein and fiber level would lead to an increased amount of water bound by the protein, thus increasing the hardness of the rice crackers. Oyster and Mussel shell is also rich in Collagen protein, which is important as building structure of human body (Xia, Zhang, Dong, & Shen, 2017). When incorporated into rice crackers, collagen protein might increase the hardness of the crackers. A similar result was demonstrated by Klunklin and Savage (2018), where incorporation of mussel powder into biscuits would increase the overall hardness of the product, although they use substitution method with the mussel powder instead of addition of the mussel powder. In other words, the biscuit containing mussel powder would possess a lower amount of wheat flour, while in this experiment addition of shellfish powder is used instead of substitution.

4.2 Colour Analysis

Table 4. Color Analysis in L* (Lightness), a*(Redness/Greenness), and b*(Yellowness) Spectrum for Crackers Sample in Both Intact Crackers Form and Powdered Form

	Crackers Form			Powder Form		
Sample	L*	a*	b*	L*	a*	b*
BL	31.54 ± 0.42 [c]	7.58 ± 0.26 [b]	-3.44 ± 0.43 [d]	41.64 ± 0.66 [e]	10.82 ± 0.18 [a]	1.75 ± 0.34 [b]
BLO	30.28 ± 0.73 [c]	6.50 ± 0.55 [c]	-1.80 ± 0.75 [c]	39.16 ± 0.87 [d]	9.32 ± 0.22 [b]	5.39 ± 1.01 [c]
BLM	31.45 ± 0.71 [c]	6.98 ± 0.31 [c]	-1.34 ± 0.78 [c]	45.08 ± 0.28 [f]	10.14 ± 0.14 [c]	8.93 ± 0.65 [d]
BRM	44.02 ± 0.84 [b]	9.35 ± 0.32 [a]	17.65 ± 0.60 [b]	57.37 ± 0.17 [c]	8.79 ± 0.08 [d]	24.50 ± 0.68 [a]
BRO	43.21 ± 0.67 [b]	9.13 ± 0.40 [a]	17.12 ± 0.56 [b]	60.41 ± 0.35 [b]	7.59 ± 0.15 [e]	25.83 ± 1.10 [a]
BR	72.13 ± 0.82 [a]	2.37 ± 0.34 [d]	23.05 ± 0.82 [a]	74.40 ± 0.10[a]	5.26 ± 0.09 [f]	24.83 ± 0.17 [a]

BL (Black Rice), BLO (Black Rice + 20% Oyster), BLM (Black Rice +20% Mussel), BR (Brown Rice), BRO (Brown Rice +20% Oyster), BRM (Brown Rice +20% Mussel)

Value: Mean±Standard Deviation

[a-f] superscripts in each column indicate significant differences ($p<0.05$)

Table 4 demonstrate that the color of the rice crackers varies between samples, depending on their ingredients. The Lightness (L*) of BL is significantly lower compared to BR ($p<0.05$), which is obvious since black rice itself possesses darker color compared to brown rice. Both greenness and yellowness of rice crackers also vary between black rice and brown rice ($p<0.05$). This difference exists due to the accumulation of the phenolic compound in black rice, especially anthocyanins, which produce dark color (Furukawa et al., 2007). When oyster or mussel powder is added to the rice crackers, the color of the rice crackers also differ significantly regardless its black rice or brown rice. The addition of shellfish powder reduces the yellowness (b*) of both BL and BR samples. For BL sample, the addition of shellfish powder increases its redness, while for BR sample, it increases the green color intensity. This result is supported by Klunklin and Savage (2018), where the addition of green-lipped mussel would boost the greenness of the bread sample significantly. For lightness (L*), the fortification process only affects the BR sample ($p<0.05$), but not BL sample ($p>0.05$), due to the low intensity of lightness for BL sample to begin with. When analyzed in powder form, more differences can be observed throughout the samples. This indicates that the heating process helps to reduce the color changes of the outside layer of the crackers, but not completely on the inner part of the crackers.

4.3 Pasting Properties

Based on **Table 5** and **Table 6,** It can be observed that the use of black rice flour instead of brown rice flour affects the pasting properties significantly in several factors. First is the peak viscosity, BL sample demonstrates higher peak viscosity compared to BR sample, regardless of flour or crackers form. The same result was also applied for the Through and Breakdown viscosity, but when it comes to Final viscosity, Setback viscosity, and Pasting temperature, BR sample shows higher values than BL. The pasting properties of a sample are related to the chemical composition of that sample, especially its starch, lipid, and protein content. In general, the lower swelling power of a rice flour would result in higher Final viscosity and Setback viscosity, while at the same time, it possesses lower Peak Viscosity and Through and Breakdown viscosity (N. Singh, Kaur, Sandhu, Kaur, & Nishinari, 2006). The swelling power of rice flour is related to the amylose content of the rice. Amylose itself possess a tight packed structure, which can inhibit the swelling capacity of the flour (Wang et al., 2016).

Table 5. Pasting Properties of Peak Viscosity, Trough Viscosity, Breakdown Viscosity, Final Viscosity, Setback Viscosity, Peak Time, and Pasting Temperature for Crackers Samples

Sample	Peak	Trough	Breakdown	Final Viscosity	Setback	Pasting Temperature(°C)
BL	1020.0±23.11^{a}	463.12±32.91^{c}	557.12±11.53^{a}	2610.70±28^{a}	2147.67±17.04^{a}	90.91±0.35^{c}
BLO	673.0±2.65^{b}	345.33±3.51^{b}	327.67±4.62^{b}	1702.33±5.13^{b}	1357.03±7.81^{b}	92.47±0.03^{b}
BLM	618.0±9.54^{c}	248.31±16.52^{a}	370.0±8.72^{c}	1662.33±7.51^{b}	1414.31±24.0^{c}	93.92±0.71^{a}
BR	451.67±7.09^{d}	190.33±3.06^{d}	261.33±4.04^{d}	1369.71±21.2^{c}	1179.38±18.11^{d}	94.72±0.36^{a}
BRO	234.33±1.53^{e}	117.33±1.16^{e}	117.21±1.01^{e}	856.33±6.81^{d}	739.04±6.08^{e}	0±0^{d}
BRM	172.33±2.31^{f}	78.09±3.61^{e}	94.33±1.53^{f}	706.67±3.06^{e}	628.67±5.69^{f}	0±0^{d}

BL (Black Rice), BLO (Black Rice + 20% Oyster), BLM (Black Rice +20% Mussel), BR (Brown Rice), BRO (Brown Rice +20% Oyster), BRM (Brown Rice +20% Mussel)

Value: Mean±Standard Deviation

a-f superscripts in each column indicates significant differences ($p<0.05$)

Other than amylose, the pasting properties also heavily rely on the amylopectin content of rice grains. Higher amylopectin content correlated positively with peak viscosity due to its ability to bind with hydrogen, which indicates higher water holding capacity of the flour (Ye, Wang, Wang, Zhou, & Liu, 2016). Consequently, higher peak viscosity would indicate lower breakdown viscosity (Hsu, Lu, Chang, & Chiang, 2015). Therefore, based on these theories, we can assume that the starch of Black rice flour possesses lower amylose content but higher amylopectin content than Brown rice flour. However, when the crackers pasting properties are observed, it demonstrates a distinct pattern with its flour counterpart. The samples using black rice all possess a higher value of all properties,

including the final viscosity and setback viscosity. It might be caused by the complexity of the crackers samples compared to flour samples. It contains different ingredients that might affect the pasting properties of the original flour sample. During the dough formation and baking process, the starch granules already under go different reactions and interactions with other ingredients, which might cause unpredictability during the RVA test.

Table 6. Pasting Properties of Peak Viscosity, Trough Viscosity, Breakdown Viscosity, Final Viscosity, Setback Viscosity, Peak Time, and Pasting Temperature for Flour Samples

Sample	Peak	Trough	Breakdown	Final Viscosity	Setback	Pasting Temp (°C)
BL	4281.30±64.50^{a}	2214.12±189.01^{a}	2067.56±126.12^{b}	4709.46±40.90^{a}	2495.22±184.31^{a}	82.73±0.71^{d}
BLO	2110.30±37.10^{d}	1228.31±55.36^{d}	882.13±20.19^{d}	3100.71±22.04^{e}	1872.30±42.34^{c}	87.12±0.03^{c}
BLM	1987.70±18.90^{c}	1077.03±43^{d}	910.74±26.54^{d}	3202.77±21.82^{e}	2125.70±37.31^{b}	87.55±0.35^{c}
BR	3359.00±40.40^{b}	1501.31±75.50^{b}	1857.73±51.76^{c}	7753.73±46.31^{b}	6252.36±81.37^{f}	87.37±0.33^{c}
BRO	1323.33±12.34^{e}	779.67±15.01^{c}	543.67±4.73^{c}	4315.32±44.36^{d}	3535.73±29.02^{e}	88.91±0.02^{b}
BRM	1372.67±15.18^{e}	759.36±34.24^{c}	613.31±30.25^{a}	4051.36±71.2^{c}	3292.16±85.50^{d}	88.92±0.06^{b}
OP	9.33±3.21^{f}	2.0±0^{e}	7.33±3.21^{e}	2.33±0.58^{f}	0.33±0.58^{g}	0±0^{e}
MP	44.33±12.86^{f}	3.1±1^{e}	41.33±12.10^{e}	5.67±0.58^{f}	2.67±0.58^{g}	94.92±0.15^{a}

BL (Black Rice), BLO (Black Rice + 20% Oyster), BLM (Black Rice +20% Mussel), BR (Brown Rice), BRO (Brown Rice +20% Oyster), BRM (Brown Rice +20% Mussel), OP (Oyster Powder), MP (Mussel Powder)

Value: Mean±Standard Deviation

a-f superscripts in each column indicates significant differences ($p<0.05$)

Both BL and BR samples display a reduction in all pasting properties when shellfish powder is added into the mixture. This might be explained by the increase in protein content of the flour and crackers after the fortification process, where protein from either oyster or mussel would absorb water, thus leaving less water to interact with the starch from rice flour. The other possible explanation is due to protein-starch interaction between protein from shellfish with starch from rice flour, which could lead to the formation of protein matrix protecting the starch from forming hydrogen bond with water (J. Singh et al., 2010). When comparing OP and MP, OP shows a 0^{o} value for pasting properties, while MP shows 94.92^{o}. This difference is a bit peculiar due to the similar nature of both ingredients. One possible explanation would be caused by the higher total starch content while at the same time shows lower protein content of MP when compared to OP **(S Table 1).**

4.4 Functional Properties

Table 7. Functional Properties of Flours Including Swelling Capacity, Water Absorption Capacity, and Oil Absorption Capacity

Sample	Swelling Capacity (ml)	Water Absorbtion Capacity (%)	Oil Absorbtion Capacity (%)
BL	14.0±0.82^{a}	110.62±9.31^{ab}	103.06±14.68^{a}
BLO	12.33±0.47^{cd}	111.94±3.77^{ab}	103.24±3.79^{a}
BLM	11.67±0.47^{bc}	116.32±7.51^{a}	92.87±5.27^{ab}
BR	13.33±0.47^{ab}	91.91±2.30^{c}	101.3±1.9^{a}
BRO	11.33±0.47^{abc}	99.32±4.69^{bc}	79.68±3.39^{bc}
BRM	11.67±0.47^{bc}	117.03±4.44^{a}	76.89±3.02^{bc}
OP	9.67±0.47^{d}	108.21±4.52^{abc}	65.128±0.94^{c}
MP	9.67±0.47^{d}	109.96±9.03^{ab}	78.75±7.04^{bc}

BL (Black Rice), BLO (Black Rice + 20% Oyster), BLM (Black Rice +20% Mussel), BR (Brown Rice), BRO (Brown Rice +20% Oyster), BRM (Brown Rice +20% Mussel), OP (Oyster Powder), MP (Mussel Powder)

Value: Mean±Standard Deviation

$^{a-d}$ superscripts in each column indicate significant differences ($p<0.05$)

The swelling capacity of rice flour can be affected by the fortification of oyster or mussel powder. However, based on **Table 7,** only BL sample has a significant reduction in Swelling Capacity when added oyster or mussel powder ($p<0.05$), while for brown rice flour, the reduction is not significant ($p>0.05$). In terms of WAC, BL sample shows a superior value compared to BR sample ($p<0.05$), but the fortification with shellfish powder does not affect the WAC of BL sample ($p>0.05$). In contrast, the addition of mussel powder significantly increases the WAC for brown rice crackers. The superiority of WAC of BL sample compared to BR might be related to the same reason for its difference in pasting properties. The phosphate group within the amylopectin carries a negative charge, forming a hydrogen bond, thus increasing its water holding capacity (Wang et al., 2016). In comparison, a high lipid content of rice flour, such as brown rice, might reduce its ability to hold water due to its hydrophobic nature (Alcázar-Alay & Meireles, 2015). In theory, the addition of shellfish powder should increase its WAC for both types of rice flour; however, only BRM shows a significant increase compared to BR ($p<0.05$). This significant jump of value is a bit peculiar, but it is explainable by the lower lipid content compared to oyster powder, which means it could absorb more water (**S Table 1**). Regarding the OAC of both flours types, there is no significant difference between BL and BR, which is in cohort with a similar experiment conducted by Kraithong et al. (2018). They conducted a test of functional properties for different genotypes of rice flour, which also includes black rice and brown rice. The result indicates there is no significant difference in Oil Absorption Index among those rice flours. In terms of fortification of rice flour, the result of our experiment is a peculiar one because it

only gives a significant decrease in OAC for Brown rice flours. One possible explanation for this could be due to the interaction between protein from shellfish powder and lipid from brown rice, which could lead to formation of protein-lipid complexes that reduce the hydrophobic components of the flour mixture (Alzagtat & Alli, 2002).

4.5 Total Starch Analysis and Starch Digestibility

The total starch content of brown rice flour and black rice flour indicates no significant differences, although BR shows lesser value compared to BL flour (**Table 8**). The minor difference might be caused by the bran layer from brown rice grains, which reduces the amount of total starch present in brown rice flour. Both OP and MP also demonstrate a similar value in total starch content, though OP shows a slightly higher value than MP. In terms of crackers product, the total starch for all samples, including fortified samples, shows no significant differences ($p>0.05$). Therefore, it can be concluded that the addition of Oyster and Mussel powder, which itself contain starch, does not significantly affect the total starch content of crackers.

Table 8. Total Starch and AUC Value (Reducing Sugar against Time Graph) for Flour and Cracker Sample

	Sample	Total Starch (g/100g)	AUC of Digested Starch
Flour	BR	66.49±0.467^{a}	196.85±12.7^{b}
	BL	71.02±0.100^{a}	264.86±0.15^{a}
	OP	12.62±0.404^{b}	142.91±12.28^{b}
	MP	8.676±0.321^{b}	162.54±12.41^{b}
Cracker	BR	46.47±2.285^{a}	199.68±2.95^{a}
	BRO	41.38±2.821^{a}	141.00±0.61^{c}
	BRM	40.83±1.741^{a}	142.90±1.91^{c}
	BL	43.37±0.425^{a}	193.01±8.28^{ab}
	BLO	43.97±9.204^{a}	166.85±10.76^{abc}
	BLM	42.30±5.352^{a}	158.96±5.68^{bc}

BL (Black Rice), BLO (Black Rice + 20% Oyster), BLM (Black Rice +20% Mussel), BR (Brown Rice), BRO (Brown Rice +20% Oyster), BRM (Brown Rice +20% Mussel), OP (Oyster Powder), MP (Mussel Powder)

Value: Mean±Standard Deviation

$^{a-c}$ superscripts in each column indicate significant differences ($p<0.05$), Flours and Crackers samples are analyzed separately

Starch digestibility is measured using a graph constructed by plotting the amount of reducing sugar released and time of digestion (**S Figure 1 and S Figure 2**). Then we evaluate the AUC to obtain the approximation of starch digestibility (**Table 8**). In terms of flour sample, the AUC of Black rice flour possesses a higher value compared to Brown rice flour ($p<0.05$), which indicates the starch granules for black rice are more prone to digestion. However, when analyzing the AUC of both brown rice and black rice in crackers form, there are no significant differences present ($p>0.05$). The high AUC value of black rice flour is an interesting part that requires further analysis. Based on research by Camelo-Méndez et al. (2016), pigmented rice containing high anthocyanin should produce a low starch digestibility product, due to its interaction with starch to produce resistant starch. After some research, there might be a possibility that the milling process of the original rice grain form into rice flour might be the explanation for this anomaly. Structural deformation of a rice grain into rice flour could affect the rate of starch hydrolysis (Tamura, Singh, Kaur, & Ogawa, 2016). The structural damage could lead to anthocyanin compounds being easier to leach out during in vitro digestion process. Due to the instability of anthocyanin in alkaline and neutral condition, inactivation of anthocyanin is imminent during the small intestine phase (Kopjar & Pilizota, 2011), thus producing a high value of starch digestibility.

The substantial difference between starch digestibility of black rice flour and brown rice flour can also be explained by using their chemical composition, especially their amylose and amylopectin content. From the discussion in the pasting properties section, we could deduce that black rice flour might contain lower amylose content than brown rice flour. The low amount of amylose in starch will lead to a lesser quantity of hydrogen bonding exist between chains, leading to producing a weaker starch crystalline structure, thus making it more susceptible to starch hydrolysis during the digestion process (Jane, Wong, & McPherson, 1997). The presence of a bran layer in brown rice flour also provides integrity and protection mechanism to starch, which has been proven to lower the rate of starch hydrolysis (Pletsch & Hamaker, 2018). This would further increase the differences between the glycemic responses of black rice flour and brown rice flour.

As mentioned above, in cracker analysis, brown rice crackers and black rice crackers do not show any significant difference between them. This might be related to the complex interaction between different components that exist in the crackers. During the process of making crackers, the starch inside the rice flour might react with the lipids and protein from other ingredients, creating starch-lipid complexes or starch-protein complexes that could increase the amount of resistant starch present (Parada & Santos, 2016). The increased amount of this resistant starch could hinder the rate of starch hydrolysis.

As in fortification of crackers with Oyster and Mussel Powder, it can be seen from **Table 8** that the fortification of Brown rice crackers provides a significant reduction of AUC value ($p<0.05$), while for

Black rice crackers it also provides reduction, although not a significant decrease ($p>0.05$). The protein from both OP and MP could create an interaction with starch from rice flour to form protein-starch complexes that could hinder the rate of starch digestibility of the crackers (J. Singh et al., 2010). The result from this experiment is supported by Klunklin and Savage (2018), where fortified biscuit with green-lipped mussel shows a significant reduction in predicted Glycemic Index. Another explanation might be related to the antioxidant properties of the shellfish powder, where the increase in antioxidant properties of fortified rice crackers could affect the activity of digestive enzymes during the digestion process (McDougall et al., 2005).

4.6 Antioxidant Assay

Using the extraction method shows that both the TPC content and FRAP value of Black rice is significantly higher than that of brown rice (**Table 9 and Table 10**). This statement is true for both flour form and cracker form. This result can be easily explained by the fact that pigmented rice contains more bioactive compounds when compared to brown rice. In the case of black rice, the high level of anthocyanin present has proven to provides high TPC value and antioxidant activity (Ziegler et al., 2018). High antioxidant properties of both OP and MP are also present in this experiment, though OP possesses a higher value in both TPC and FRAP value compared to MP. When incorporated into crackers, both fortified black rice crackers and brown rice crackers show higher content of TPC, but for FRAP value, only brown rice crackers demonstrate significant improvement. From these results, we can assume that the bioactive compounds from oyster or mussel powder could be successfully incorporated into crackers production without losing their antioxidant properties. The same result could be observed from an experiment by Klunklin and Savage (2018), where the addition of green-lipped mussel powder into biscuit had significant improvement to its Total Phenolics content. When comparing both TPC and FRAP results, it shows

Table 9. Total Phenolic Content (TPC) of Flour and Cracker Samples Extract From In Vitro Digestion and Extract from Ethanol Extraction

	Sample	*In Vitro* Digestion		Extraction
		0H	2H	
Flour	BL	0.26±0.01[b]	0.37±0.02[b]	1.68±0.02[b]
	BR	0.17±0.01[a]	0.21±0.01[a]	0.39±0.04[a]
	OP	0.68±0.01[d]	0.94±0.01[c]	4.50±0.10[c]
	MP	0.55±0.02[c]	1.02±0.05[d]	3.75±0.36[d]
Crackers	BL	0.16±0.04[a]	0.21±0.02[c]	1.99±0.11[b]
	BLO	0.18±0.01[a]	0.33±0.02[b]	2.48±0.18[a]
	BLM	0.15±0.02[a]	0.40±0.03[a]	2.40±0.16[a]
	BR	0.08±0.01[a]	0.21±0.01[c]	0.74±0.01[c]
	BRO	0.15±0.02[a]	0.31±0.01[b]	1.68±0.14[b]
	BRM	0.14±0.01[b]	0.30±0.01[b]	1.70±0.11[b]

BL (Black Rice), BLO (Black Rice + 20% Oyster), BLM (Black Rice +20% Mussel), BR (Brown Rice), BRO (Brown Rice +20% Oyster), BRM (Brown Rice +20% Mussel), OP (Oyster Powder), MP (Mussel Powder)

Value: Mean±Standard Deviation

[a-c] superscripts in each column indicate significant differences ($p<0.05$), Flours and Crackers samples are analysed separately

Asides from evaluating the extract from flour and cracker samples, we also perform an analysis on the extract from starch *in vitro* digestion process. From this evaluation, several interesting points can be observed. First is a different pattern can be observed for Black Rice and Brown Rice when they are in flour forms or in crackers form. The extract from flour samples during in vitro digestion shows that BL possess higher TPC and antioxidant properties than BR; however, when the flours are incorporated into crackers, the TPC content of BL and BR is the same. For FRAP value, BL crackers still show a significantly higher value than BR at the beginning of digestion, but after 2 hours digestion process, the FRAP value becomes the same for both. These peculiar results suggest that food characteristics can influence the bioactive compounds and how they are released during the digestion process (Pinacho, Cavero, Astiasarán, Ansorena, & Calvo, 2015). A complex interaction between these bioactive compounds with food components, such as protein, fiber, lipid, and starch, could be responsible for this unpredictability (Bouayed, Hoffmann, & Bohn, 2011)

Table 10. Ferric-Reducing Antioxidant Power (FRAP) of Flour and Cracker Samples Extract From In Vitro Digestion and Extract from Ethanol Extraction

	Sample	*In Vitro* Digestion		Extraction
		0H	2H	
Flour	BL	1.56±0.05[b]	1.61±0.12[b]	11.20±0.47[b]
	BR	0.87±0.10[c]	0.77±0.09[c]	2.31±0.06[c]
	OP	2.60±0.09[a]	2.38±0.05[a]	15.54±0.09[a]
	MP	1.60±0.09[b]	2.17±0.08[a]	14.64±0.39[b]
Crackers	BL	0.93±0.12[a]	1.25±0.09[a]	11.43±0.44[a]
	BLO	0.96±0.00[a]	1.12±0.04[a]	11.28±1.55[a]
	BLM	0.73±0.14[ab]	1.29±0.09[a]	11.98±0.18[a]
	BR	0.69±0.09[b]	1.13±0.09[a]	2.88±0.11[c]
	BRO	0.84±0.04[ab]	1.20±0.10[a]	5.78±0.52[b]
	BRM	0.89±0.04[ab]	1.13±0.09[a]	6.47±0.19[b]

BL (Black Rice), BLO (Black Rice + 20% Oyster), BLM (Black Rice +20% Mussel), BR (Brown Rice), BRO (Brown Rice +20% Oyster), BRM (Brown Rice +20% Mussel), OP (Oyster Powder), MP (Mussel Powder)

Value: Mean±Standard Deviation

[a-c] superscripts in each column indicate significant differences ($p<0.05$), Flours and Crackers samples are analysed separately

The second important thing to discuss is how the TPC and FRAP value changes during in vitro digestion process. From observing **Table 9** and **Table 10**, we can conclude that, in general, the digestion process would increase both TPC and FRAP values, regardless of if it is flour or cracker samples. A previous experiment conducted by Thuengtung et al. (2018) resulted in similar outcomes, where the FRAP value of rice grain would increase during in vitro digestion. However, it is important to note that in their experiment, a whole intact grains sample is used, rather than a milled flour sample. It has been discussed that the use of whole-grain samples could better preserve the bioactive compounds during the digestion process compared to milled grain samples, but the process of using whole grain requires alteration to the *in vitro* digestion process. In their experiment, Thuengtung et al. (2018) perform more than 24 hours of digestion process for evaluating the whole grain samples. Other reports regarding antioxidant properties during in vitro digestion suggest that the FRAP value would decrease during digestion process (Li, Deng, Liu, Loewen, & Tsao, 2014). This contradicting result might be related to the difference in the sample used in the experiment, in which Li et al. (2014) used tomato as their sample. As it is mentioned above, different food characteristics would produce different outcomes. The increase of TPC and FRAP value might be explained by the breakdown of the phenol-protein compound during digestion process. Based on experiment by Tamura et al. (2016), the digestive enzyme might prefer to interact with this phenol-protein complexes, which when broken down, makes phenolic compounds easier to be released.

Chapter 5

Conclusion

In conclusion, implementing different types of rice flour for making rice crackers would influence the outcomes in both physical characteristics and nutritional components. Brown rice flour would produce crackers that are softer in texture and brighter in color compared to those using black rice flour. The pasting properties of brown rice flour demonstrate lower peak viscosity, but it produced higher final viscosity compared to black rice flour, which might be related to its higher amylose content. Brown rice flour also excels in terms of hindering the rate of starch digestibility compared to black rice, although it does not show significant difference after they have been processed into crackers. However, black rice is superior in terms of antioxidant properties and TPC content when compared to brown rice, which largely due to its high anthocyanin content.

The fortification of rice crackers with either oyster or mussel powder also shows significant changes in some areas of the crackers. For brown rice crackers, the fortification process significantly increases the hardness, though it does not affect the fractubility. The addition of these shellfish powder also successfully lowers the starch digestibility rate for both brown rice crackers and black rice crackers, although only brown rice crackers show significant decrease. Lastly, the antioxidant activity and TPC content for both crackers types are significantly improved by the addition of oyster or mussel powder. However, the antioxidant properties evaluation during in vitro starch digestion still poses some uncertainties which should be further investigated in future experiments.

Chapter 6

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Appendix A

Supplementary Tables and Figures

A.1 Comparison between Oyster and Mussel Powder

Supplementary Table 11. Chemical Composition of Oyster Powder and Mussel Powder

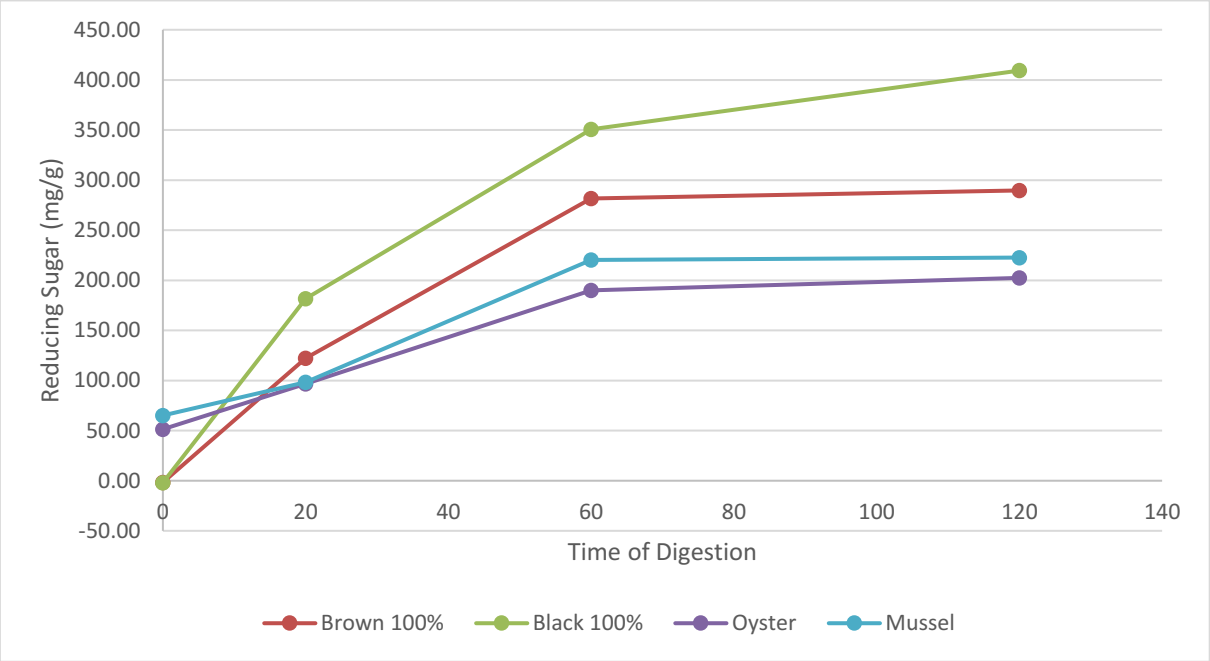
Sample	Crude Protein (g/100g)	Total Starch (g/100g)	Ash (g/100g)	Lipid (g/100g)	Carbohydrate (g/100g)
Oyster Powder	51.7±0.05*	11.56±0.19*	8.21±0.05*	9.11±0.06*	25.92±0.15*
Mussel Powder	49.3±0.04*	15.75±0.53*	19.42±0.04*	6.01±0.08*	21.10±0.12*

Value: Mean±Standard Deviation

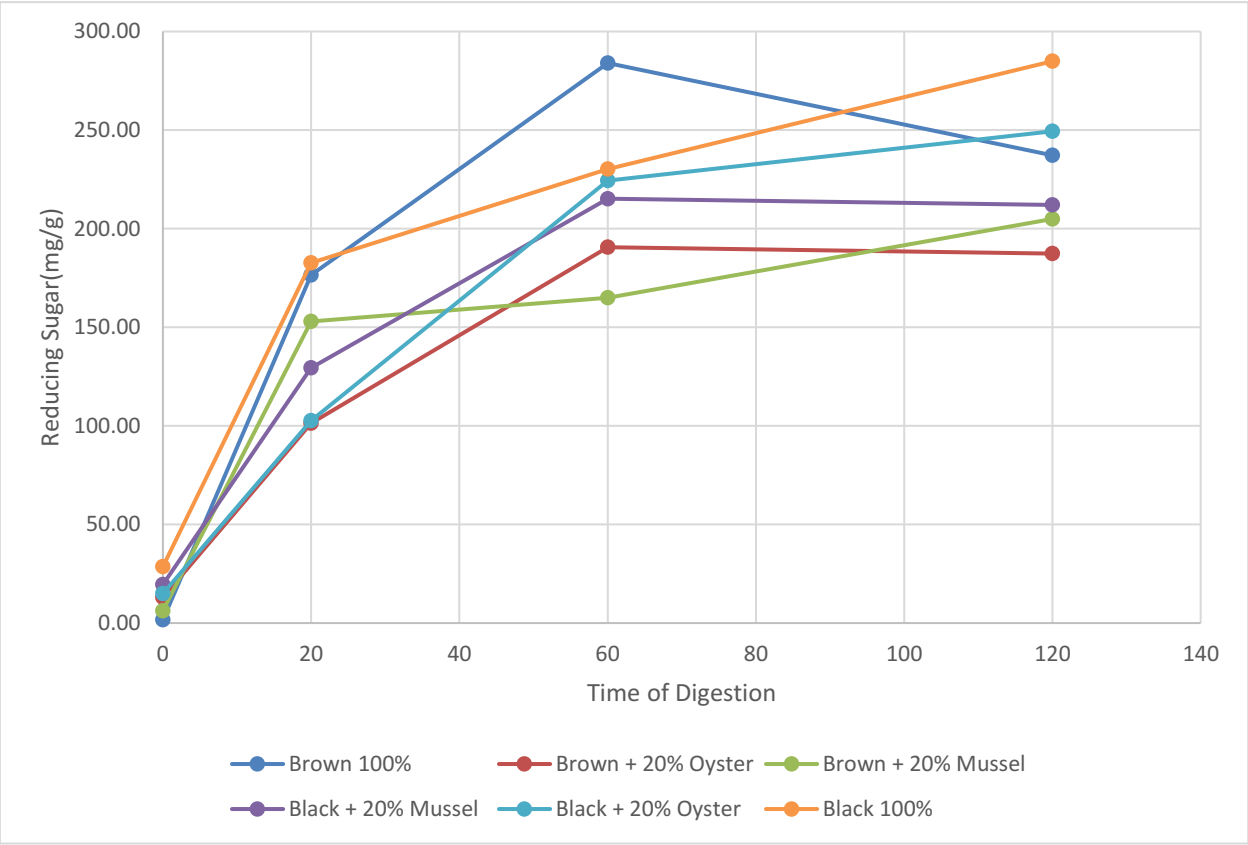
* indicates significant difference is present

Notes: Supplementary Table 1 is obtained from experiment conducted by unpublished research paper by Lincoln University PhD student, Ashley Sui (2020)

A.2 Predicted Glycaemic Responses between Samples



S Figure 1. Reducing Sugars Released during In Vitro Digestion (2 hours) for Flour Sample



S Figure 2.Reducing Sugars Released during In Vitro Digestion (2 hours) for Cracker Sample